

WHAT IS CLAIMED IS:

1. A method for altering the cleavage specificity of a
5 Type IIG restriction endonuclease, the Type IIG restriction
endonuclease characterized by a cleavage domain adjacent to
a methylase domain, the methylase domain located adjacent
to a specificity domain, the method comprising:

(a) ligating a first DNA sequence and a second DNA
10 sequence to form a fusion DNA , wherein

(i) the first DNA sequence comprises a DNA
segment encoding a catalytic domain and an N-terminal
portion of a methylase domain of a first Type IIG restriction
endonuclease, and

15 (ii) the second DNA sequence, comprises a DNA
segment encoding a specificity domain and a C-terminal
portion of a methylase domain of a second Type IIG
restriction endonuclease;

such that the ligation occurs between sequences
20 encoding the methylase domain; and

(b) transforming a host cell with the fusion DNA for
expressing a Type IIG restriction endonuclease with altered
cleavage specificity.

25 2. A method according to claim 1, wherein step (a) further
comprises: introducing a mutation into the cleavage domain to
enhance the viability of the transformed host cell.

3. A method according to claim 1, wherein the sequence corresponding to the N-terminal portion of the methylase terminates in a methylase conserved motif selected from motifs X, I, II, III, IV, V, VI, VII or VIII.

5

4. A method according to claim 1, wherein the sequence corresponding to the C-terminal portion of the methylase terminates in a methylase conserved motif.

10

5. A method according to claims 3 or 4 wherein the conserved motif is selected from motifs X, I, II, III, IV, V, VI, VII or VIII wherein the N-terminal portion and the C-terminal portion of the methylase are non-overlapping.

15

6. A method according to claims 3 or 5, wherein the sequence corresponding to the N-terminal portion of the methylase motif terminates between the sequence encoding motif III and NPPY in motif IV.

20

7. A method according to claim 1, wherein ligation occurs by means of a linker sequence attached to each of the N-terminal portion of the methylase domain and the C-terminal portion of the methylase domain on the first and second DNA segment.

25

8. A method according to claim 1, wherein the fusion DNA encodes an active methylase domain.

9. A method according to claim 1, wherein the first and second Type IIG endonucleases have defined cleavage and recognition sites.

5 10. A method according to claim 1, wherein the first Type IIG endonuclease has a defined cleavage and recognition site and the second Type IIG endonuclease is characterized by a bioinformatic search of a microbial sequence database.

10 11. A method for forming a non-natural, functional Type IIG restriction endonuclease, wherein the Type IIG restriction endonuclease is characterized by a functional cleavage domain, a functional methylase domain and an altered functional specificity domain, compared with a natural form of the functional Type IIG endonuclease, comprising :

15 (a) inserting into a DNA encoding the methylase domain or the specificity domain of the natural form of the functional Type IIG endonuclease, a mutation or a nucleic acid linker sequence for inactivating optionally the cleavage domain and inactivating (i) the functional methylase domain and the

20 specificity domain or (ii) the functional methylase domain or the functional specificity domain;

(b) ligating to the DNA at the mutation or at the linker, a DNA encoding (i) a portion of the methylase and specificity

25 domain or (ii) a portion of the methylase or specificity domain to form a fusion DNA; and

(c) transforming a host cell having a marker for detecting a colony expressing a non-natural functional Type IIG restriction endonuclease.

5 12. A method according to claim 11, wherein the mutation is positioned within a conserved motif in the methylase domain.

10 13. A method according to claim 11, wherein the mutation is a deletion at a 5'- end of the DNA encoding the specificity domain.

14. A method according to claim 11, wherein the mutation is a deletion within the specificity domain.

15 15. A method according to claim 11, wherein the linker is a transposon mediated linker insertion sequence.

20 16. A method according to claim 11, wherein the linker contains a restriction endonuclease cleavage site which is unique within the DNA encoding the restriction endonuclease.